

# **MicroRNA Expression Profile and DNA Damage Response in Cultured Human Fibroblasts in Space**

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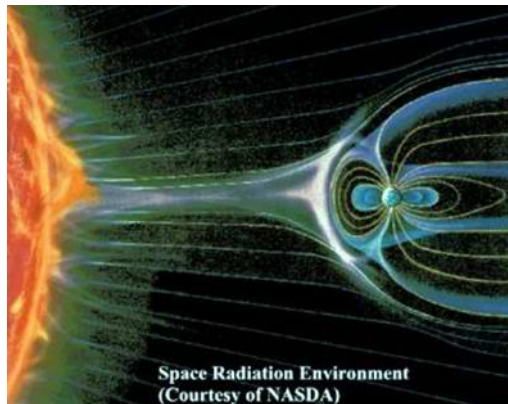
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## Questions to be Addressed

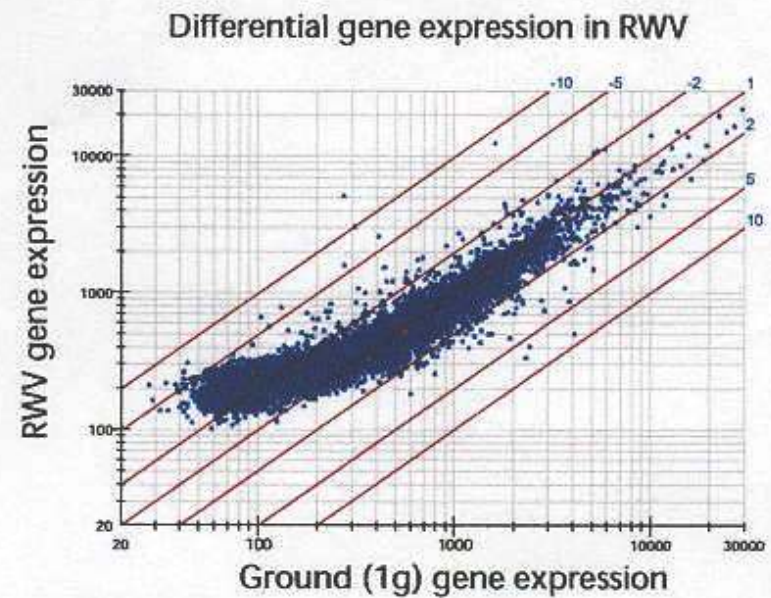
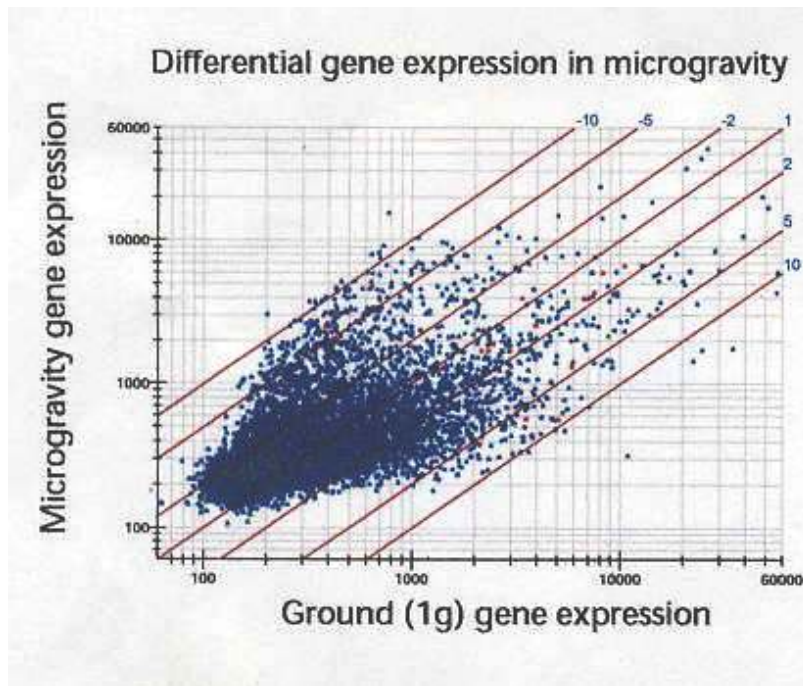
Does spaceflight influence RNA and miRNA expressions in non-dividing cultured cells?

Does microgravity affect cellular responses in living organisms to space radiation exposure?

Does microgravity and other spaceflight factors affect cellular responses to DNA damages?



# Gene expression changes in space

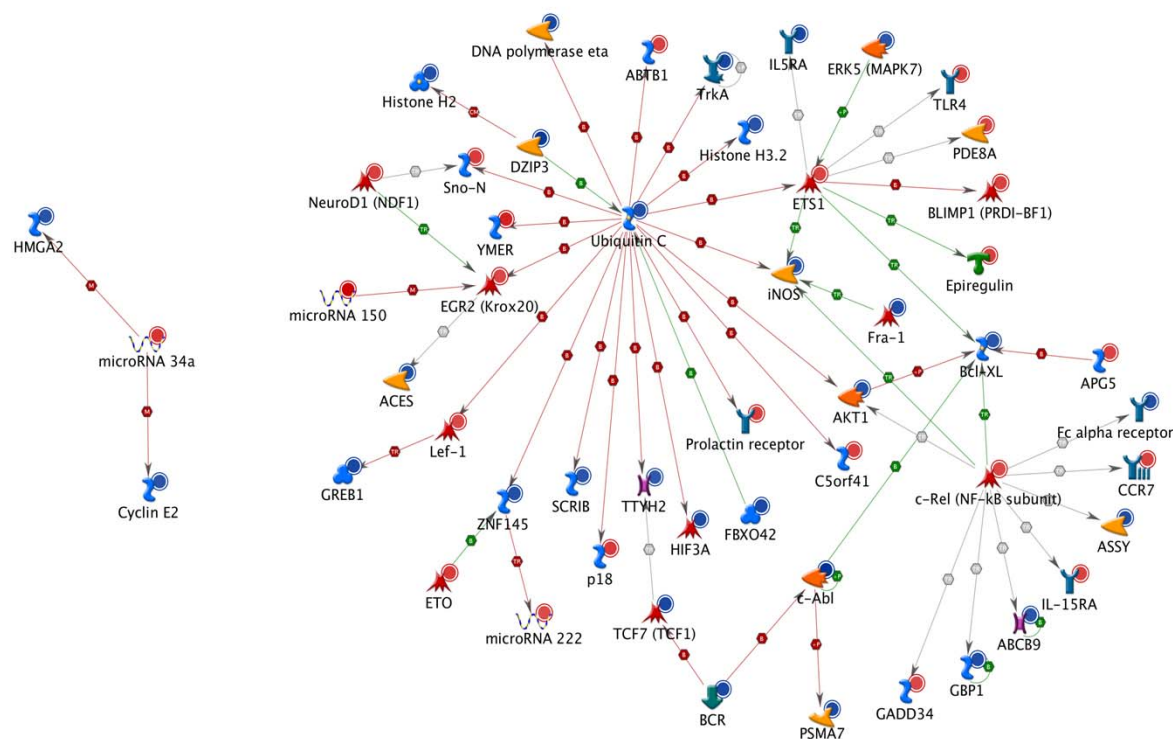


Hammond et al. Nature Medicine 1999

# Effects of Simulated Microgravity on Expression Profile of MicroRNA in Human Lymphoblastoid Cells<sup>\*[S]</sup>

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The direct interaction analysis showed several projected networks with c-Rel, ETS1 and Ubiquitin C as key factors. Several genes showed direct interactions with miRNAs that were found to be altered in simulated microgravity environment. Seven genes cyclin E2, HMG2, EGR2, ZNF145, Ubiquitin C, ETS1 and c-Rel were subjected to validation analysis using Quantitative Real-time PCR.



Chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated in vitro with low-LET radiation  
(Wu et al. Phys. Med. 2001)

Mission: STS-103

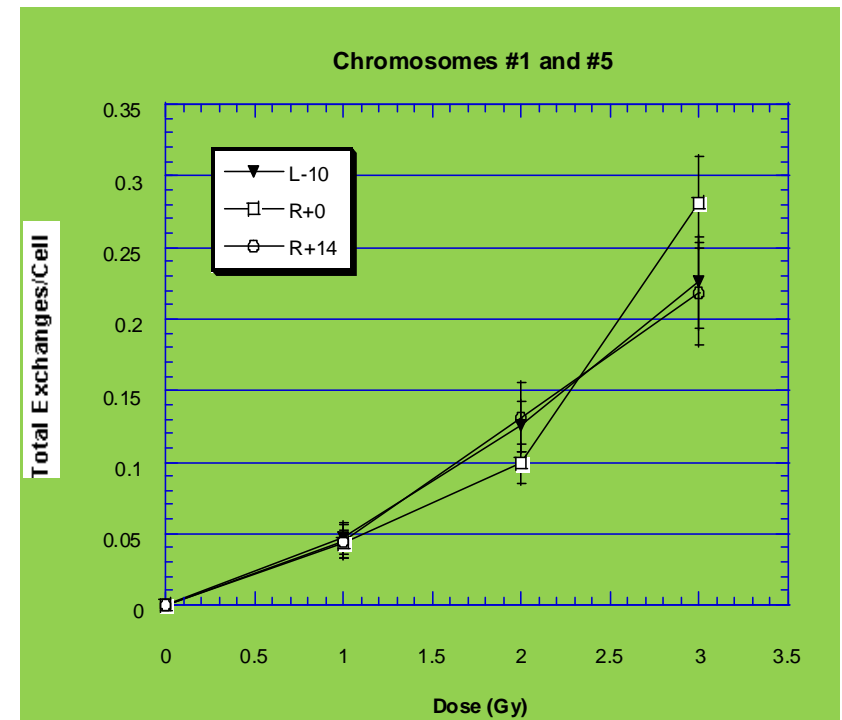
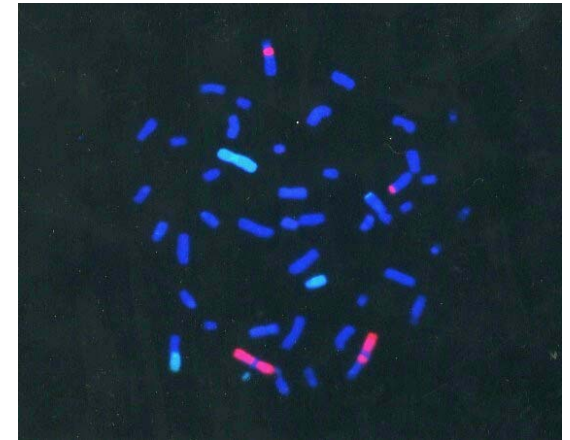
Duration: 8 days

Blood draw schedule: L-10, R+0 and R+14

Irradiation: Whole blood was collected from the astronaut and irradiated with gamma rays

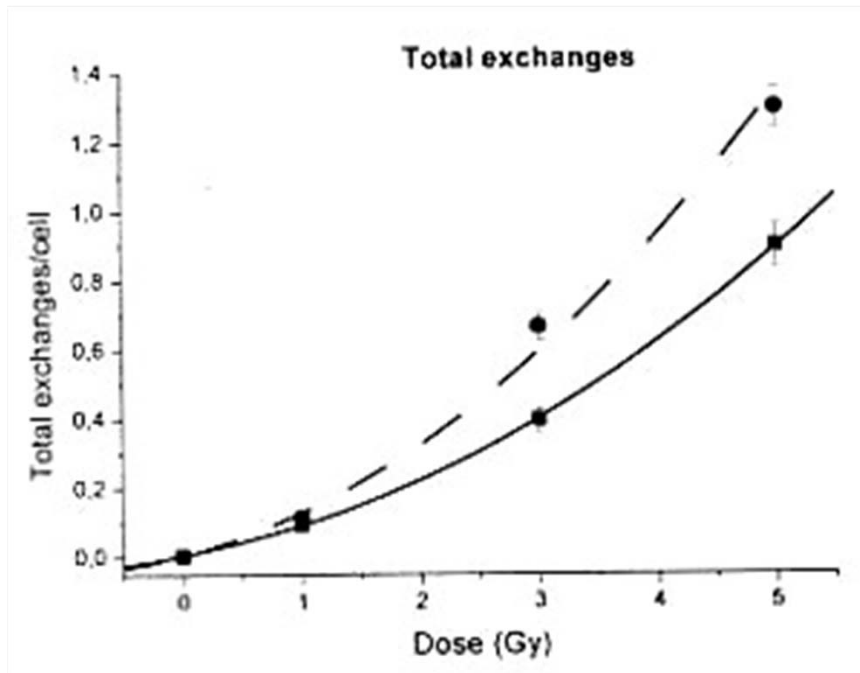
Chromosome analysis: Chromosomes #1 and #5 were painted.

Conclusion: No differences in radiosensitivity were found.



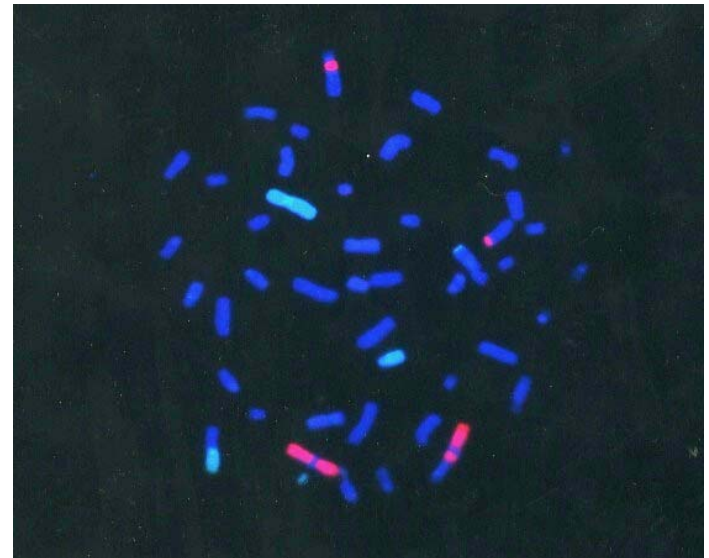


## Greco et al. Adv. Space Res. 2003



Significant difference was found in the dose response curve between the pre- and post-flight samples.

- Cosmonaut's blood samples were collected 3 days after landing
- Samples were exposed to X-rays



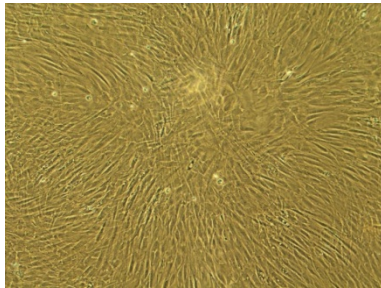
### MICRO-7 FLIGHT EXPERIMENT

- Investigate changes of RNA and miRNA expressions in G1 human fibroblast cells in space
- Investigate the cellular response to bleomycin-induced DNA damages in Ge human fibroblast cells in space

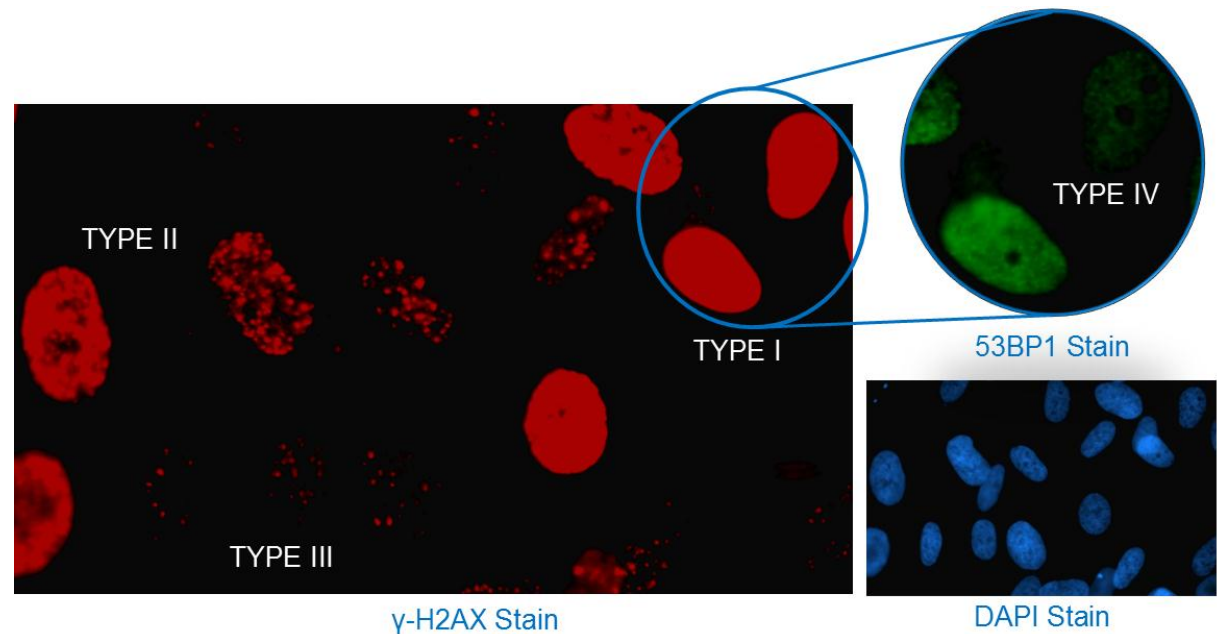


Our Micro-7 experiment was launched in April, 2014

Human fibroblasts

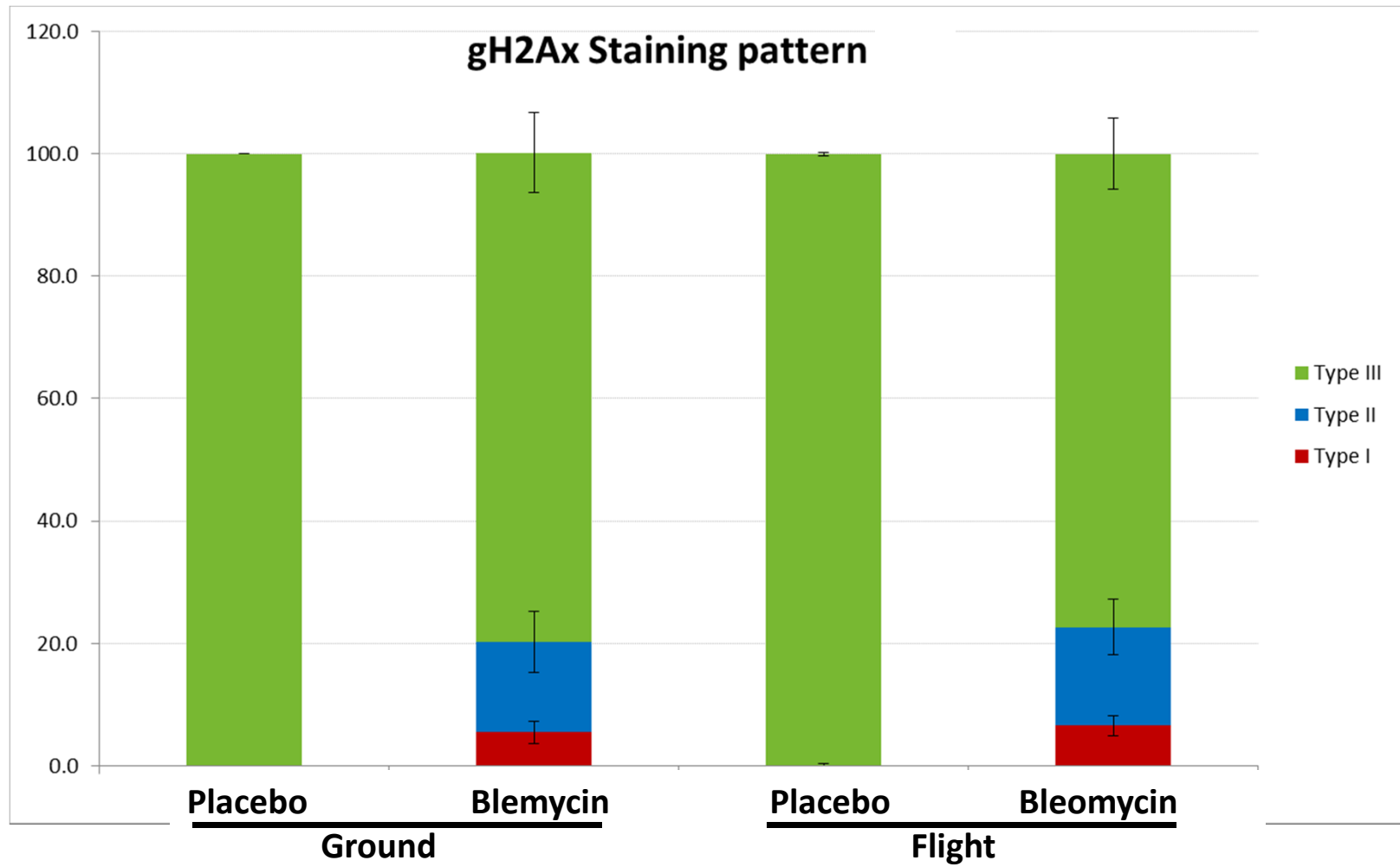


BioCell from Bioserve

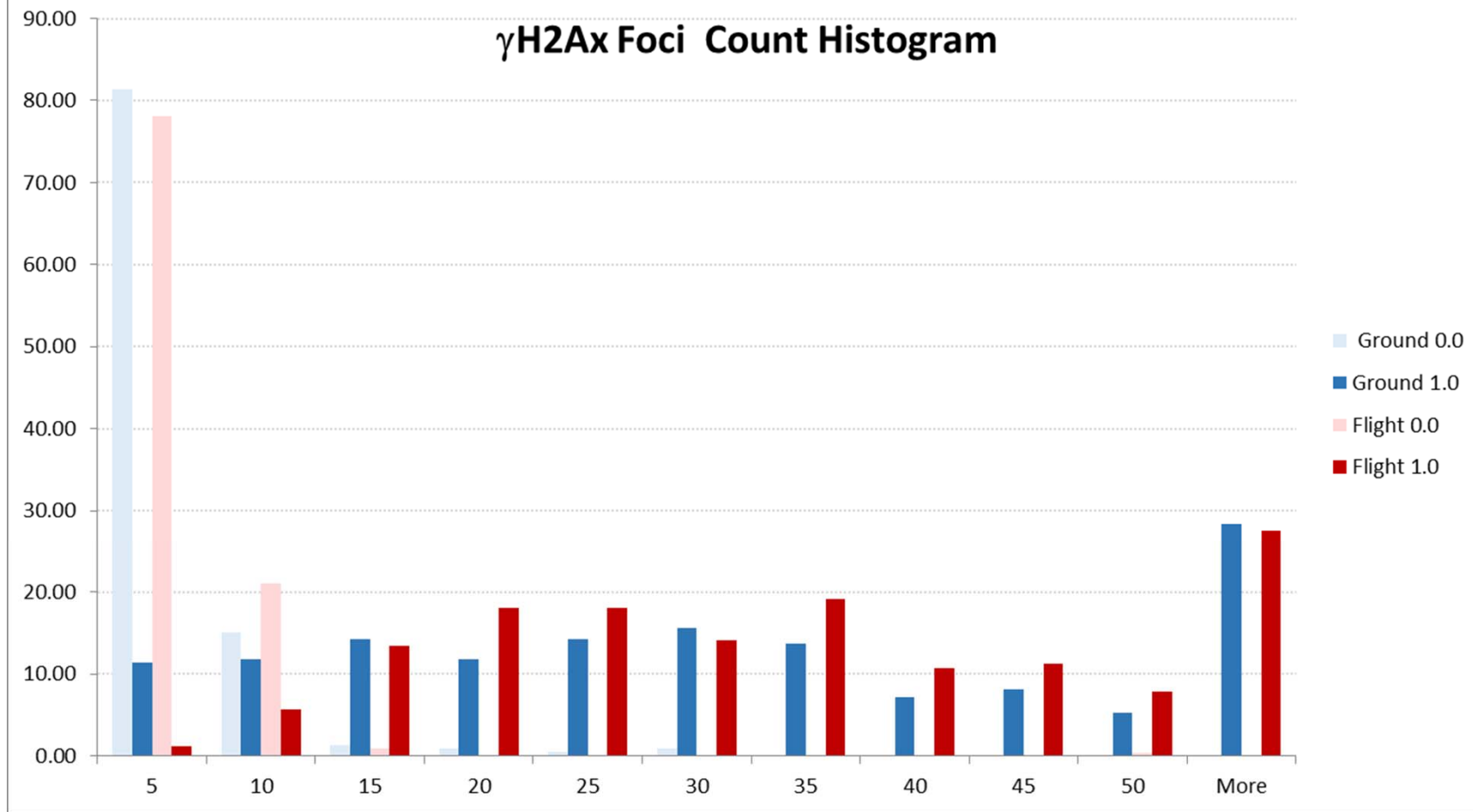


Damage in human fibroblasts will be measured by the phosphorylation of a histone protein H2AX after bleomycin treatment.

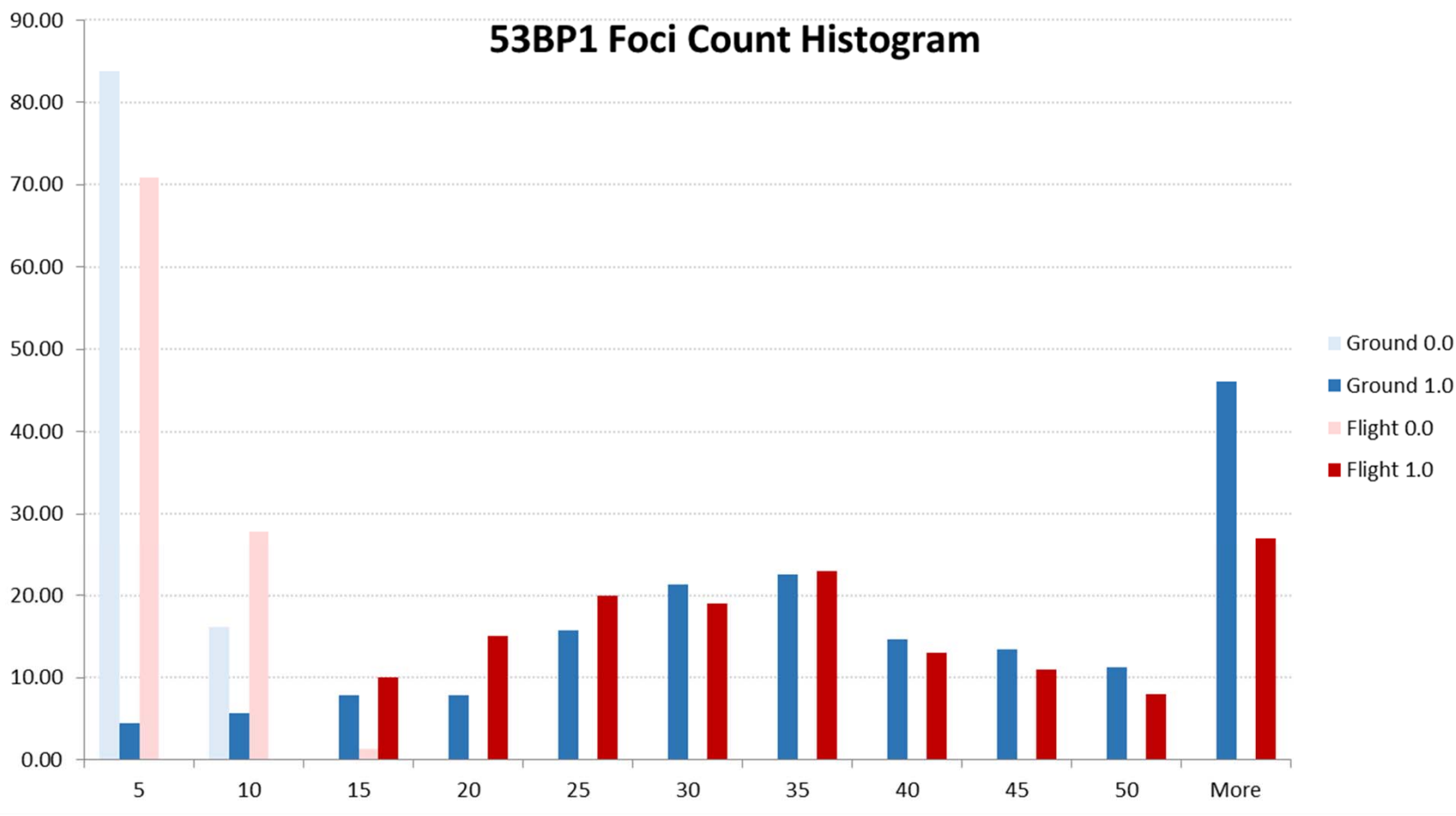


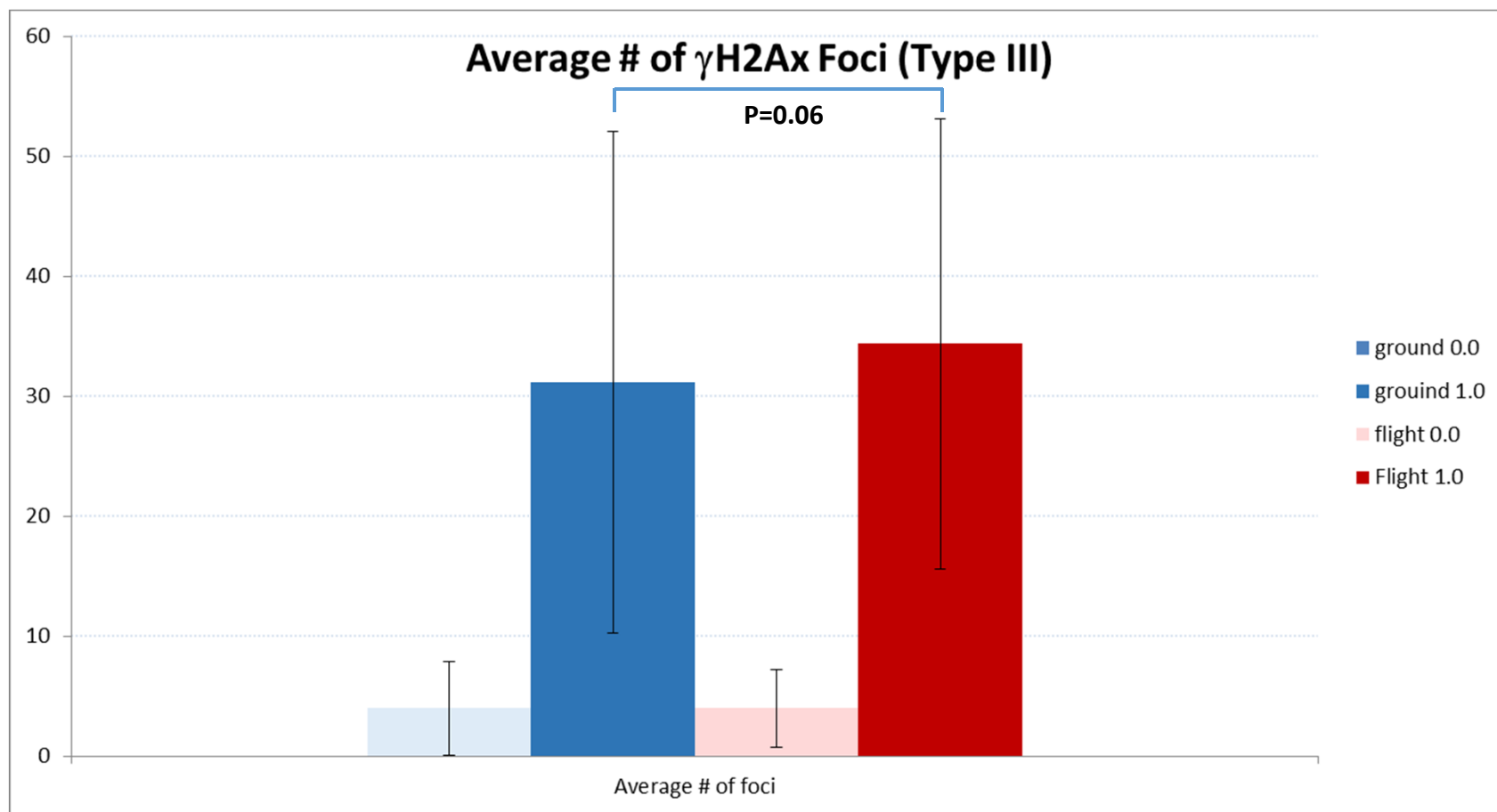


**$\gamma$ H2Ax Foci Count Histogram**

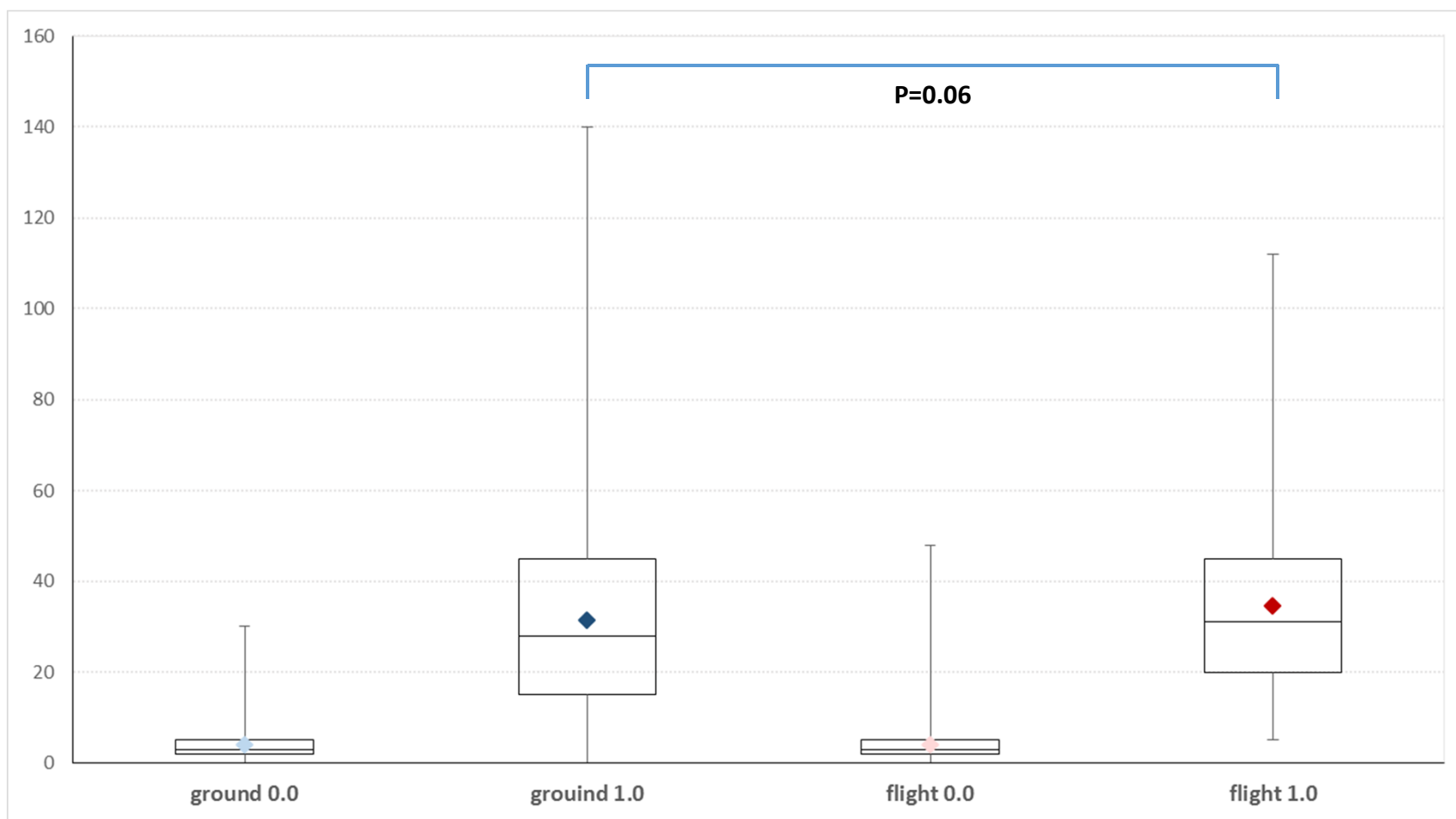


53BP1 Foci Count Histogram



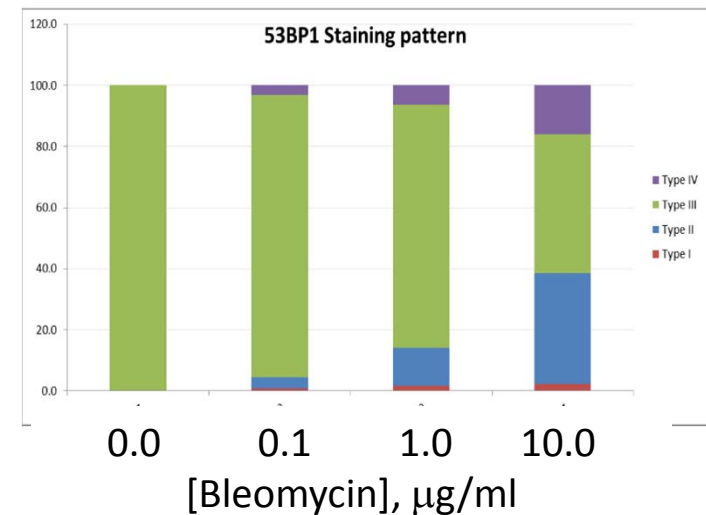
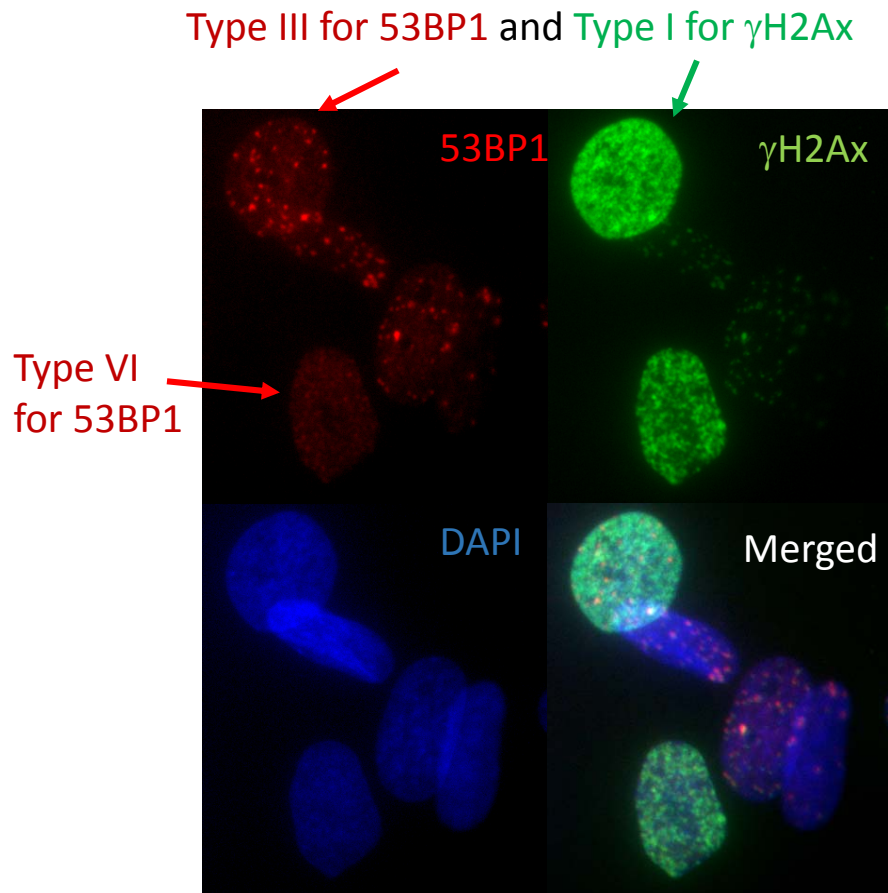


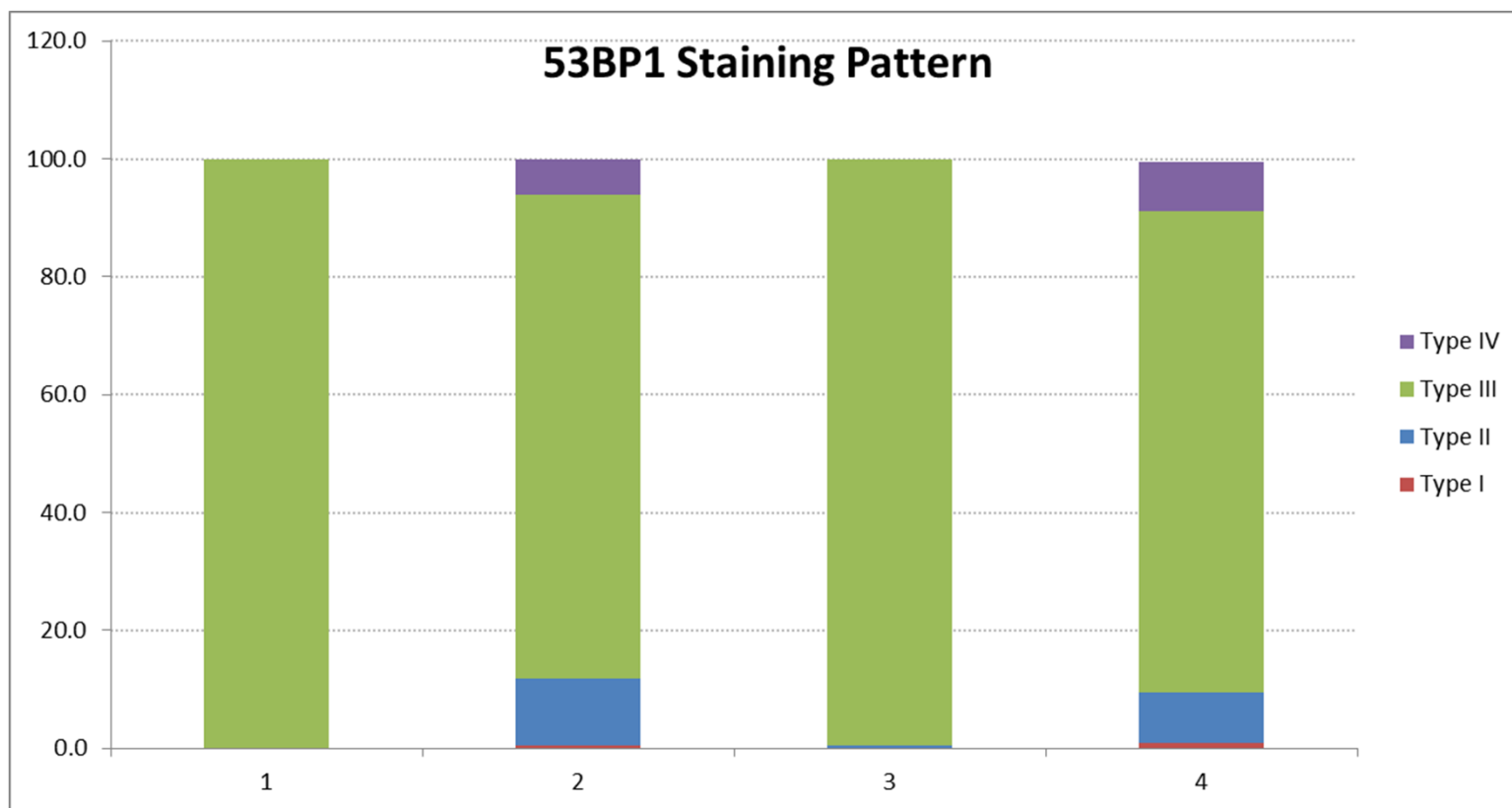
# Average # of gH2Ax Foci (Type III)

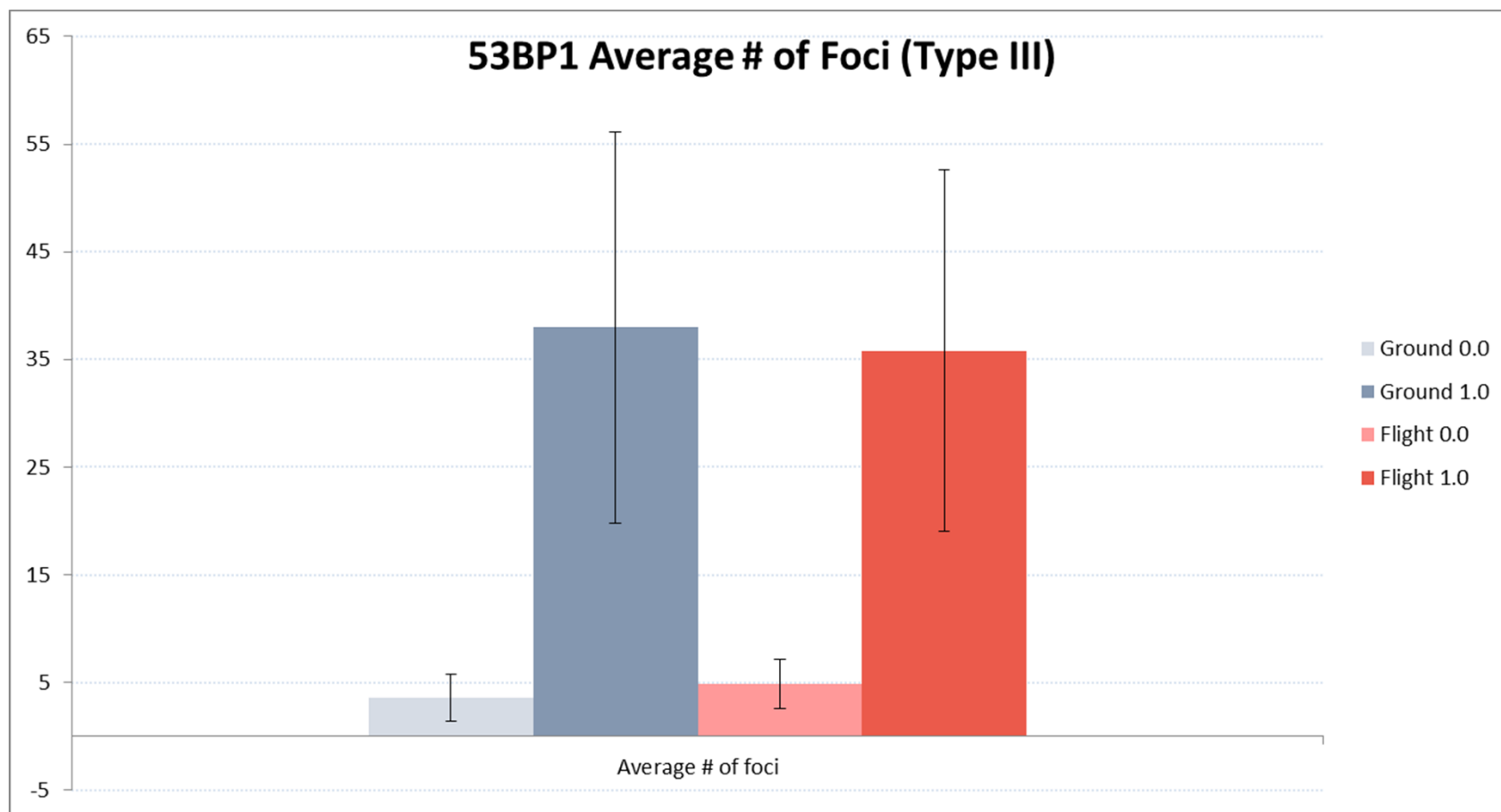


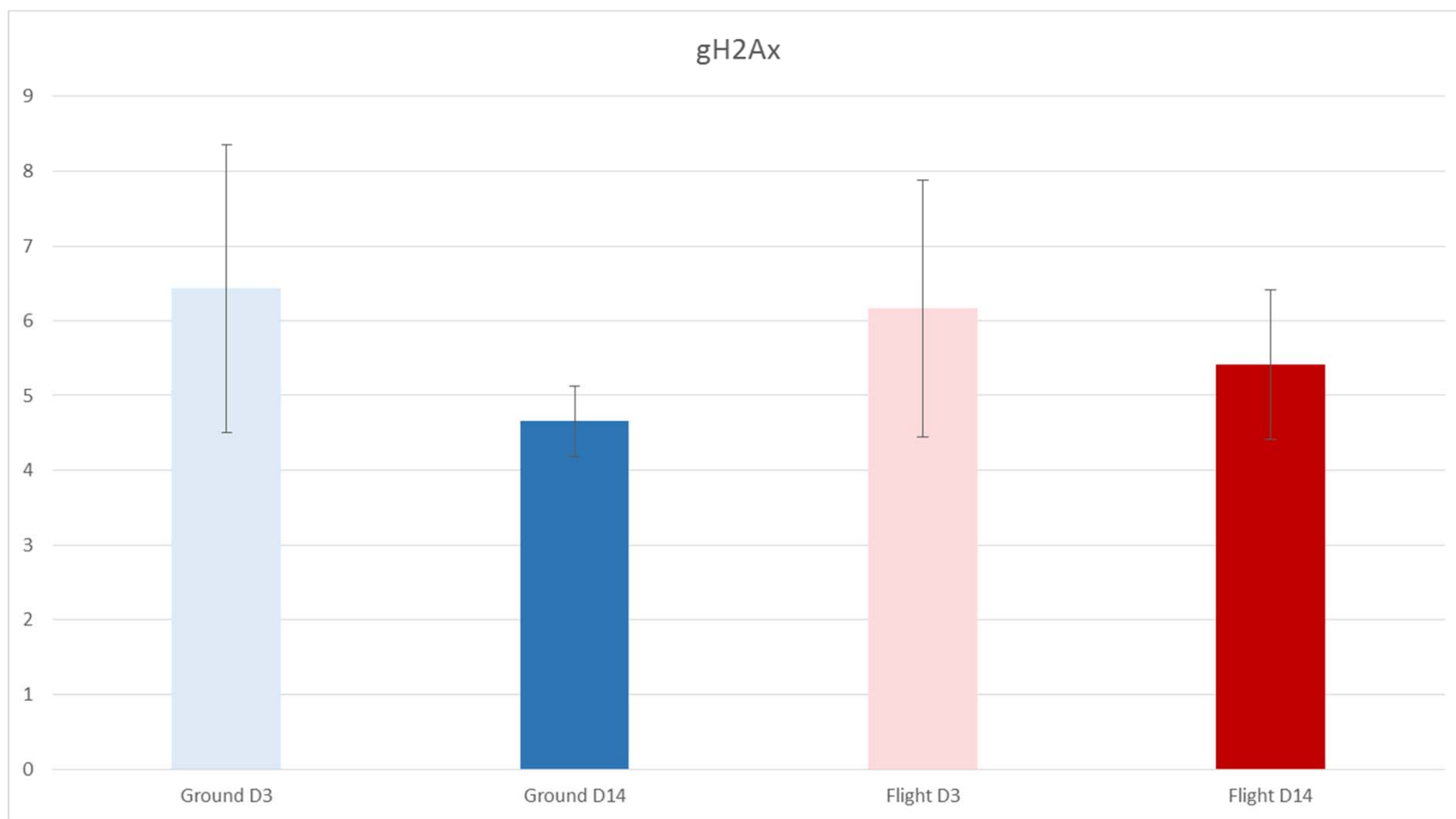


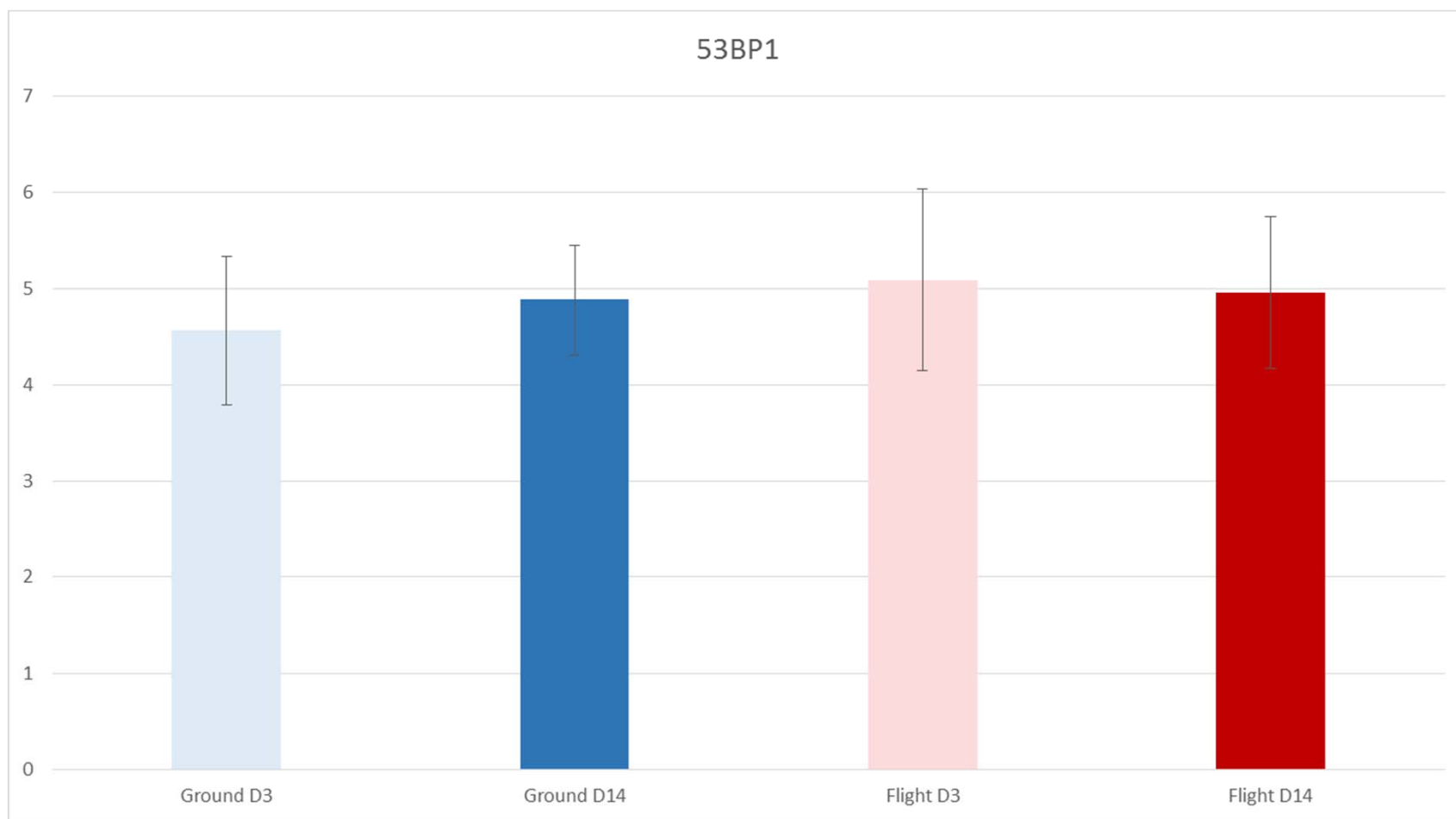
# Quantification of DNA Damages with 53BP1 Immunofluorescence Staining Patterns and Foci Counts



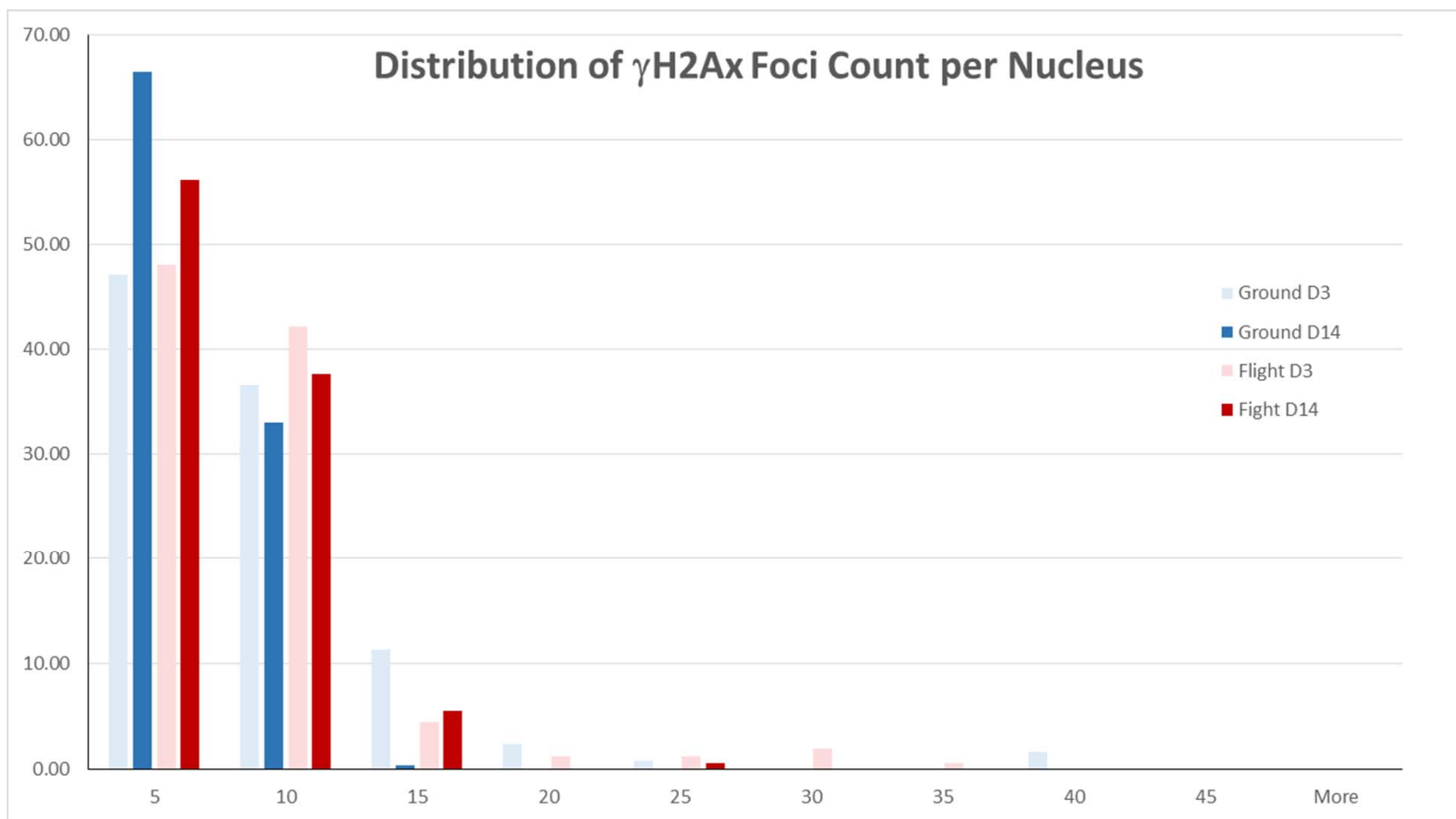


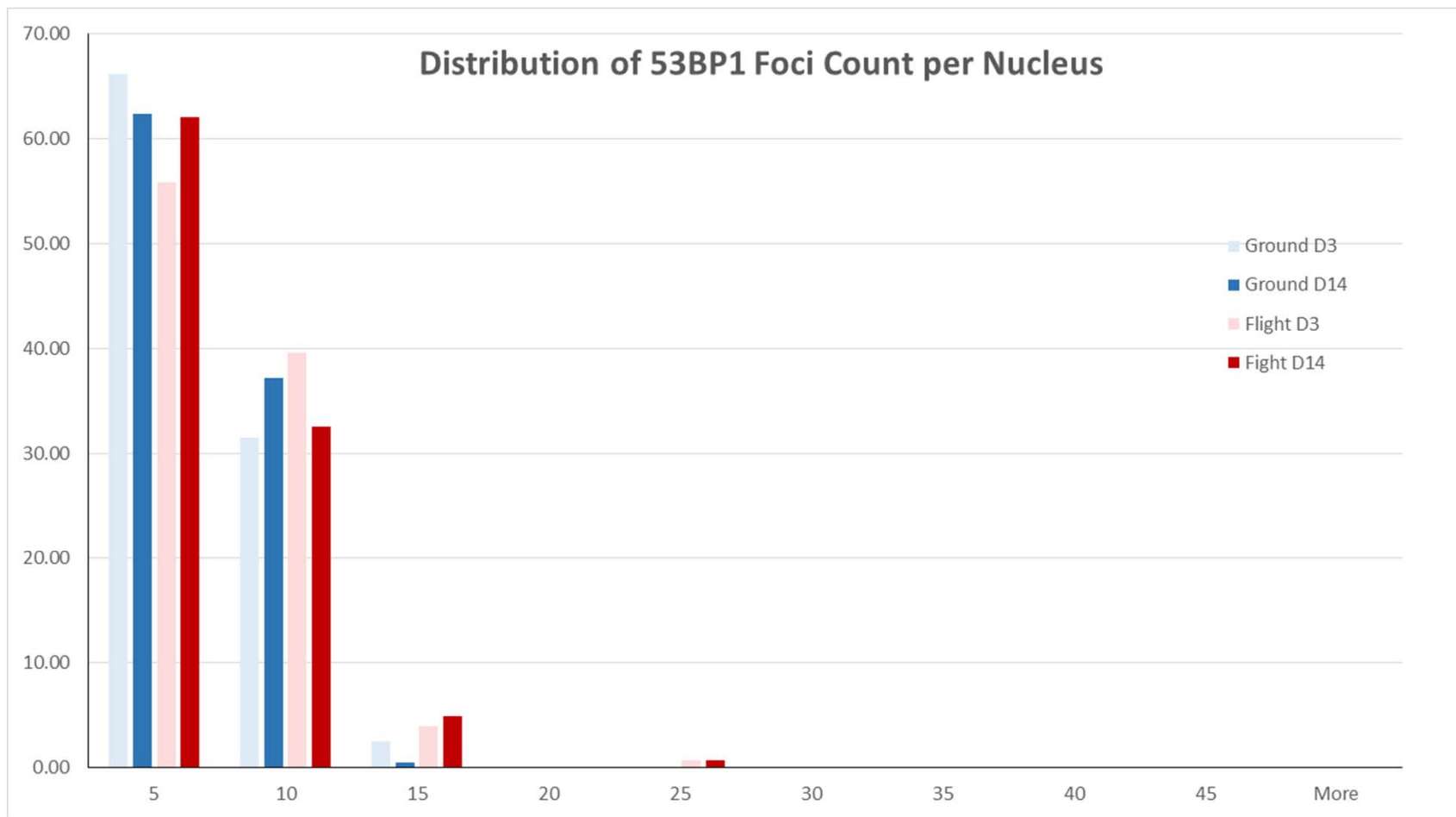




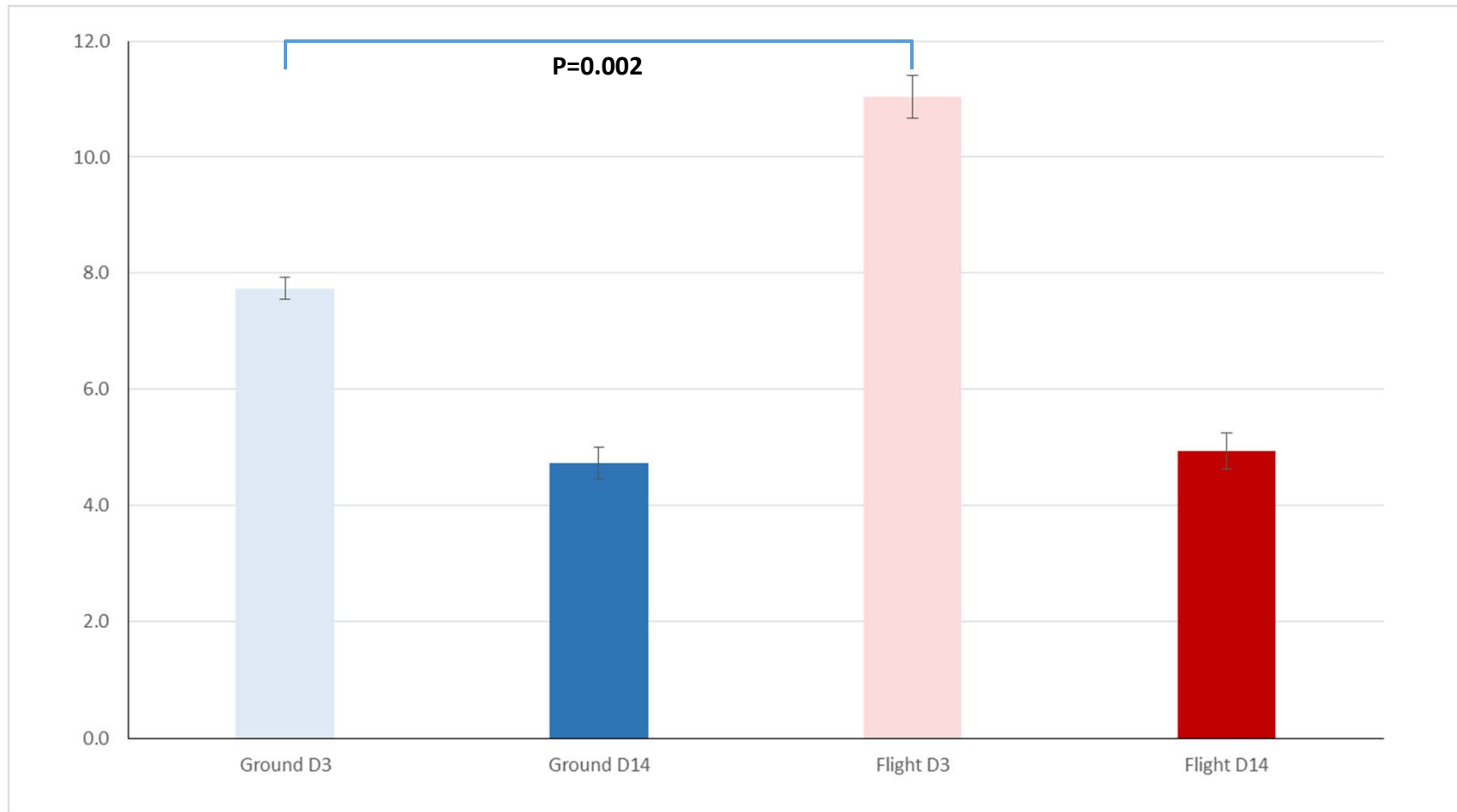




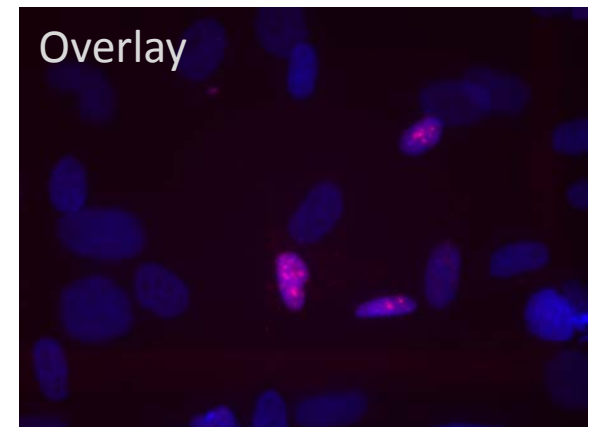
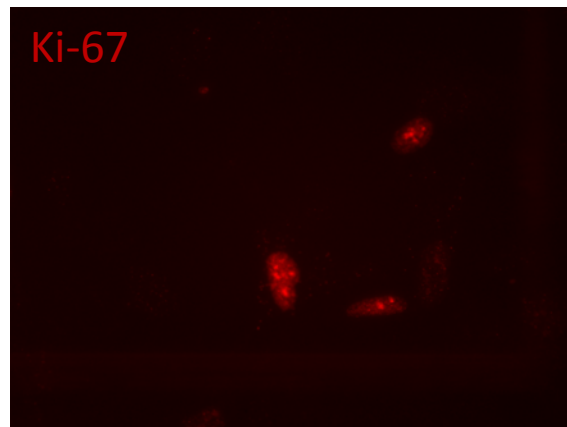
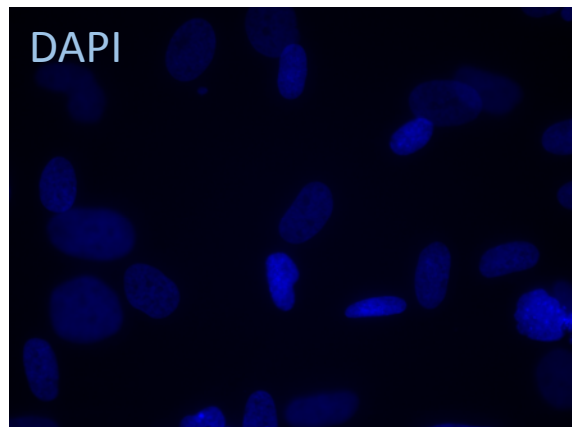




## Percent of Ki-67 Positive staining cells.



## Cell proliferation marker – Ki67



# CONCLUSIONS

- Human fibroblast cells in the G1 phase of the cell cycle were flown on ISS for 3 and 14 days. Microarray analysis of gene expressions did not show a significant difference between the flight and the ground samples for either of the days.
- The gene expression patterns were significantly different between the 3 and 14 day samples, due potentially to the slow growth of the cells.
- On Day 3 after reaching the orbit, the cells were exposed to bleomycin to induce DNA damages. The degree of damages, as measured with immunohistochemistry staining for the induction of gamma-H2AX and 53BP1, did not show a significant difference in the response between the flight and ground samples.



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